PB83-171280

Determination of 2,3,7,8-TCDD (Tetrachlorodibenzo-p-Dioxin) in Industrial and Municipal Wastewaters, Method 613. Part 1 Development and Detection Limits

(U.S.) Environmental Monitoring and Support Lab.
Cincinnati, OH

1983

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3
EPA-600/D-83-020	PUBLISHED PAPER	PB83-171280

4. TITLE AND SUBTITLE	5. REPORT DATE
The Determination of 2,3,7,8-TCDD in Industrial and Municipal Wastewaters. Method 613 Part 1 - Development and Detection Limits	1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) D.L. Foerst, J.E. Longbottom, R.J. Wesselman, et al.	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Monitoring and Support Laboratory U. S. Environmental Protection Agency 26 W. St. Clair Street Cincinnati, Ohio 45268	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U. S. Environmental Protection Agency 26 W. St. Clair Street Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/06

15. SUPPLEMENTARY NOTES
American Clinical Society Symposium on Chlorinated Dioxins & Dibenzofurans in the Total Environment Park 1. 9/13-17, 1982, Kansas City

16. ABSTRACT

A convenient method for water and wastewater samples is presented that will determine 2,3,7,8-TCDD concentrations to a Method Detection Limit (MDL) of 2 ng/L. The method uses solvent extraction of the aqueous sample followed by capillary column gas chromatography/mass spectrometry using Selected Ion Monitoring. The capillary column (Silar 10C or equivalent) is isomer specific for 2,3,7,8-TCDD.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	26
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

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SPRINGFIELD, VA. 22161

EPA-600/D-83-020
PUBLISHED PAPER

The Determination of 2,3,7,8-TCDD in Industrial and Municipal Wastewaters, Method 613 Part 1 - Development and Detection Limits

A.S. Wong, M.W. Orbanosky, and P.A. Taylor
California Analytical Laboratories, Inc.
Sacramento, California 95824

C. R. McMillin, R.W. Noble, and D. Wood
Monsanto Research Corporation, Dayton Laboratory
Dayton, Ohio 45407

J.E. Longbottom, D. L. Foerst, and R. J. Wesselman
U.S. Environmental Protection Agency
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

Abstract

A method for the determination of ng/L concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,6,7-TCDD) in industrial and municipal wastewaters is presented. The method is based on solvent extraction of the aqueous sample, clean-up on silica gel or alumina columns, and capillary column gas chromatography/low resolution mass spectrometry (selective ion monitoring) determination of 2,3,7,8-TCDD. Method Detection Limits of 2 ng/L are achieved using an isomer specific capillary column.

INTRODUCTION

On June 7, 1976, the U.S. EPA agreed to a consent decree establishing a list of toxic compounds for which industrial and municipal wastewater effluent limitations were to be developed and promulgated by June 30, 1982. These specific toxic compounds, known as the "Priority Pollutants," included 114 organic compounds.[1] The final regulations concerning effluent limitations will be applied to point sources through the National Pollutants Discharge

Elimination System (NPDES). As such, analytical methods for the 114 organic compounds had to be developed and made available for the NPDES program in a relatively short period of time.

In 1977, the Environmental Monitoring and Support Laboratory located in Cincinnati, Ohio (EMSL-CI) began a contract program to develop analytical methods for the priority pollutants. The 114 organic compounds were subdivided into 13 groups of chemically related compounds. Contracts for the development of analytical methods were awarded to five laboratories which eventually produced Methods 601 thru 613. These methods were published *in toto* in the Federal Register on December 3, 1979 [2] as proposed rulemaking under section 304(h) of the Clean Water Act.

Method 613 for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is unique in that it alone contains one target compound and requires the use of high resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS) during the assay step. As a result of public comment and recent advances in analytical procedures, the final version of Method 613 is different from the initial version which appeared in the Federal Register. The following discussions will detail the development of both the initial and current versions of Method 613.

INITIAL METHODS DEVELOPMENT

LITERATURE REVIEW -- The contractor for the development Method 613, California Analytical Laboratories, Inc., Sacramento, California, performed a literature review at the beginning of the contract in the Spring of 1978 to determine the current state of the art for 2,3,7,8-TCDD analyses. The litera-

ture review found four papers relevant to the determination of 2,3,7,8-TCDD in herbicide and pesticide formulations.[3-6] Common to those procedures was the use of alumina columns during the cleanup step to separate 2,3,7,8-TCDD from free acid and ester herbicides or chlorophenols. 3,4,5 All four papers described the use of packed column gas chromatography during the assay step. Electron capture detectors were used in the earlier procedures.[3,4] A flame ionization detector [5] and a mass spectrometry techniques [6] were used in the more recent work in which instrumental detection limits of 30 ppb and below 10 ppb respectively were reported.

The use of glass capillary columns was prevalent in the more recent papers which included reports concerning the utility of OV-101, OV-17 [7] and Silar 10C [8] liquid phases. Other recent advances in the mode of detection for detection 2,3,7,8-TCDD included the use of chemical ionization (CI) and negative ion chemical ionization (NICI) mass spectrometry.[9,10]

The method to be developed for the NPDES program had restrictions, namely the method should not involve techniques or equipment not routinely found in or readily available to a commercial analytical laboratory. This ruled out requiring the use of CI or NICI and high resolution mass spectrometry (HRMS) during the assay step. Glass capillary columns coated with OV-101 and OV-17 or their equivalents (SP-2100 and SP-2250) were commerically available but columns coated with Silar 10C were not.

The restrictions applied to the methods development permitted electron capture detector screening. Any positive extract would be submitted for confirmation and quantitation by GC/MS. This was done to keep costs in line with the other NPDES methods being simultaneously developed. The screening approach did not permit adding isotopically labeled 2,3,7,8-TCDD to the

original sample, thus the state of the art reflected in the early version of Method 613 was somewhat compromised by these restrictions.

EXPERIMENTAL

EXTRACTION -- The pH of the aqueous sample and choice of extracting solvent can affect the recovery through the extraction step. Therefore, a series of experiments were performed to determine if the traditional solvent system for pesticide analysis (15% methylene chloride in hexane) would be favored over the more convenient solvent system of 100% methylene chloride.

The efficiency of each solvent system was evaluated in triplicate at three pH values pH = 2, 7, and 10. One-liter aliquots of reagent water were adjusted to the desired pH by adding 0.1 N HCl or 0.1 N NaOH as appropriate. The aliquots were then spiked with 100 ng of 2,3,7,8-TCDD and serially extracted with three 60-mL portions of the extracting solvent. The organic phases were dried over sodium sulfate and concentrated to 10 mL using Kuderna-Danish (K-D) techniques. Toluene was added (1.0 mL), and the samples were concentrated to 5.0 mL using K-D and finally concentrated to 1.0 mL using nitrogen blow down. The results are summarized in Table 1. Neither the solvent system nor the pH of the original sample exhibited a deletorious effect upon recovery. Recoveries were uniformly above 90% in all cases. Fewer emulsion problems were encountered using 100% methylene chloride when compared to using 15% methylene chloride in hexane. Since the organic phase is on the bottom of the separatory funnel when 100% methylene chloride is used, the potential for exposure is reduced because the number of manipulations involved are less when compared to using 15% methylene chloride in hexane. These

considerations led to choosing 100% methylene chloride as the extracting solvent. No pH adjustment was judged necessary to optimize recovery.

TABLE 1

EXTRACTION EFFICIENCY: Recovery Versus Solvent System and pH

Dosing Level	Solvent System		Percent Recovery[a]		
(ng/L)	% Hexane	% Methylene Chloride	pH=2	pH=7	pH=10
100	85	15	93 ± 3.7	100 ± 6.8	95 ± 7.7
100	0	100	92 ± 6.2	92 ± 5.5	91 ± 5.5

a) average of three replicates

REMOVAL OF ACIDIC AND BASIC COEXTRACTANTS -- The literature review indicated a consensus need for washing the organic extract with aqueous base and acid prior to any further concentrations. Thus, no further investigations of this step were performed. After the extraction from water, the method specified extracting the 180 mL methylene chloride phase twice with 100 mL of 1.0 N sodium hydroxide, twice with 100 mL of 1.0 N sulfuric acid, and three times with 100 mL of reagent water. These manipulations involve relatively cumbersome sample processing techniques because of the less dense aqueous phase was to be discarded.

CONCENTRATION AND SOLVENT EXCHANGE -- Both methylene chloride and hexane solutions of 2,3,7,8-TCDD are concentrated at various steps of the procedure. Table 2 summarizes the recovery of 2,3,7,8-TCDD when different solvent systems were concentrated from 200 mL to 1.0 mL using K-D procedures followed by nitrogen blowdown procedures. The recovery of 2,3,7,8-TCDD was quantitative for 100% hexane, 15% methylene chloride in hexane, and 100% methylene chloride.

TABLE 2

CONCENTRATION EFFICIENCY: Recovery Versus Solvent System

Dosing Level	Solvent System		Percent Recovery[a]
(ng)	% Hexane	% Methylene Chloride	200mL to 1.0mL
100	100	0	97 ± 5.8
100	15	85	103 ± 2.5
100	0	100	104 ± 7.8

a) average of three replicates

SAMPLE PRESERVATION -- The effluents from treatment processes are often chlorinated as a final polishing step in the treatment process. Chlorine, which is a strong oxidizer, can react with analytes when they are stored even for short periods of time. The temperature and pH during storage can also exhibit effects on the stability of the sample prior to extraction. The effect of the four variables: chlorination, pH, temperature, and time, was studied with one series of experiments. Sufficient sodium hypochlorite was added to reagent water to give a 10 ppm level of free residual chlorine. The pH was adjusted to pH = 2, 7, or 10. Samples were stored in glass containers for 14 days at either $4^{o}C$ or $25^{o}C$. The spiking level was 1000 ng/L of 2,3,7,8-TCDD. The results are summarized in Tables 3 and 4. The GC/MS system was used during the assay step in these series of experiments since the chlorinated samples gave interference problems with the packed column-electron capture detector system.

The chlorinated water samples gave uniformly lower recoveries of 2,3,7,8-TCDD after 14 days, regardless of the temperature at which the samples were stored. There was also some loss exhibited for the non-chlorinated samples. A further series of experiments was performed to

investigate the effect in chlorinated water. A 1000 ng/L solution of 2,3,7,8-TCDD at pH = 7 was divided into 90-mL aliquots in separate 100 mL volumetric flasks and sodium hypochlorite was added to give 10 ppm of free residual chlorine. The flasks were capped, stored at 25°C and extracted with 1.0 mL of hexane on days 0,3,6,9,12, and 15. A plot of recovery versus time is given in Figure 1. The rate of disappearance was not as large as that found in the earlier study, and showed a 10% loss on the sixth day and 22% loss on the fifteenth day. By requiring that samples be analyzed by the seventh day after collection, the studies indicated there is no need to add a chemical preservative to guard against a serious loss of 2,3,7,8-TCDD due to chlorination.

TABLE 3

RECOVERY FROM CHLORINATED WATER: pH Versus Time Versus Temperature

Dosing Level		Percent Recovery[a]		
		Day 0	Day 14	
(ng/L)	pH		4°C	25°C
1000	2	91 ± 10	44 ± 6.2	51 ± 6.0
1000	7	82 ± 8.2	50 ± 5.5	38 ± 8.6
1000	10	84 ± 5.7	43 ± 8.2	32 ± 8.6

a) average of three replicates

TABLE 4

RECOVERY FROM NONCHLORINATED WATER: pH Versus Time Versus Temperature

Dosing Level		Percent Recovery[a]		
		Day 0	Day 14	
(ng/L	pH		4°C	25°C
1000	2	81 ± 9.0	66 ± 8.0	78 ± 10.9
1000	7	91 ± 9.1	68 ± 7.6	77 ± 5.9
1000	10	98 ± 8.1	74 ± 4.6	79 ± 7.2

a) average of three replicates

SAMPLE CLEANUP -- Four open-column, gravity-fed chromatographic systems were evaluated for separating 2,3,7,8-TCDD from coextractants in complex samples. The systems tested were alumina, silica gel, Florisil, and silica gel/charcoal.

Alumina cleanup columns were prepared from Fisher grade A 540 80/200 mesh neutral alumina that was activated at 130°C for 24 hours. A 30 cm x 10 mm ID glass column was filled to a height of 15 cm and topped with 1 cm of sodium sulfate. After equilibration with 50 mL of benzene, the 1 mL hexane solution of 100 ng of 2,3,7,8-TCDD was quantitatively transferred to the column, which was then eluted with 50 mL of 3% methylene chloride in hexane, 50 mL of 20% methylene chloride in hexane and 50 mL of 100% methylene chloride. The 2,3,7,8-TCDD was only found in the 20% methylene chloride fraction and exhibited an average recovery of 86% ± 9% (Table 5). This column is useful for separating polychlorinated biphenyls (PCBs), 2,4,5-trichlorophenol, and hexachlorophene from 2,3,7,8-TCDD.

The silica gel cleanup column was adapted from that described earlier.[6] Silica gel (14 gr of 100/200 mesh Davidson 923) was packed into a 30 cm x 10 mm ID glass column and equilibrated with 50 mL of 20% benzene in hexane. A 1.0 mL aliquot containing 100 ng of 2,3,67,8-TCDD was then added to the column. Another 40 mL of 20% benzene in hexane was collected, concentrated and analyzed. The mean recovery was 90% ± 8% for the triplicate experiments (Table 5). This column is useful for separating 2,3,7,8-TCDD from chlorophenoxy acid herbicides since these herbicides are retained by the silica gel.

EPA procedures for pesticides generally recommend Florisil column cleanup procedures. Therefore, the Florisil technique in Method 608, The

Determination of Chlorinated Pesticides and PCBs, was evaluated. Thirty percent of the 100 ng of 2,3,7,8-TCDD eluted from the Florisil column in the 6% diethylether/hexane fraction and 62% eluted in the 15% diethylether/hexane fraction (Table 5). This procedure does not afford a useful separation. Most of the pesticides listed in Method 608, including all the PCBs, elute in the 6% diethylether/hexane fraction. This procedure was not included in Method 613.

California Anaytical Labs was also developing methodology for determining 2,3,7,8-TCDD in sludges and sediments. Some of the environmental samples on hand provided an exceptional challenge for the standard cleanup procedures. The extract was still a dark oily intractable mixture after sequential alumina and silica gel cleanup was performed. A silica gel/charcoal cleanup procedure was developed in response to these samples and applied to aqueous samples. The adsorbent was a mixture of 1 gram of 50/200 mesh activated coconut charcoal (Fisher Scientific 5-690-A) and 140 grams of 100/200 mesh silica gel (Davidson 923). A 5 mm ID disposable Pasteur pipette was packed to give a 5 cm bed of the mixed adsorbent. After equilibrating this microcolumn with 5 mL of hexane, the 1.0 mL aliquot of 10 ng or 100 ng of 2,3,7,8-TCDD was added to the column. The first fraction, 10 mL of hexane, was discarded. The 2,3,7,8-TCDD eluted in the next 10 mL of benzene. Recovery was low (40 to 50%) but the precision was adequate (Table 5). This procedure was a great aid in simplifying the sediment and sludge extracts so it was included in the early version of Method 613. It was to be used only after alumina and silica gel procedures were used and the extract was still intractable and needed further cleanup.

TABLE 5

Efficiency of Various Cleanup Procedures

Column	Spike Level (ng)	Fraction Collected	Percent Recovery
Alumina	100[a]	20% methylene chloride in hexane	86 ± 9
Silica Gel	100[a]	20% benzene in hexane	90 ± 8
Florisil	100[b]	6% diethyl ether in hexane	30 ± 3
		15% diethyl ether in hexane	62 ± 5
Silica Gel/charcoal	10[a]	100% benzene	46 ± 4
	100[a]	100% benzene	43 ± 5

a) average of three replicates
b) average of five replicates

CHROMATOGRAPHY -- The packed glass columns and glass capillary columns that were evaluated were chosen because of availability and applicability to other EPA procedures. The packed glass columns were 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh Gas Chrom Q, 1.8 m x 4 mm ID, 3% OV-17 on 100/120 mesh Gas Chrom Q, 1.8 m x 4 mm ID; and 1% SP-2250 on 100/120 mesh Supelcoport, 1.2 m x 2 mm ID. All were adequate and gave instrumental detection limits in the range of 10 to 20 pg per injection using either electron capture detection or selected ion monitoring mass spectrometry. The mixed phase column was recommended because it is the primary analytical column in Method 608 and should be readily available in laboratories performing routine pesticide analyses.

The capillary columns evaluated were 30 m x 0.25 mm ID borosilicate glass columns coated with SP-2250 or SP-2100, the equivalent of the columns cited in reference 7. SP-2250 was recommended because it gave better resolution between 2,3,7,8- and 1,2,3,4-TCDD. The latter isomer could be

used as a surrogate compound as an internal standard. Figure 2 shows the performance expected for the SP-2250 capillary column after a splitless injection of 20 pg of 2,3,7,8-TCDD when the MS was operated in the Selected Ion Monitoring (SIM) mode.

EVALUATION OF THE INITIAL METHOD -- The initial Method 613 which appeared in the Federal Register is outlined as a flow chart in Figure 3. After screening by GC/ECD or HRGC/ECD, the presence of 2,3,7,8-TCDD is confirmed and the concentration measured using HRGC/MS. The following criteria were to be achieved to confirm the presence of 2,3,7,8-TCDD.

1) The retention time of the native 2,3,7,8-TCDD is the same as that of the authentic standard.

2) The isotope ratio (320/322) must agree to within ±10% of the authentic standard.

3) m/z 320 and m/z 322 must exhibit a simultaneous maximum.

The contractor evaluated this method using 1.0 liter aliquots of reagent water, industrial wastewater, and municipal wastewater that were spiked with 2,3,7,8-TCDD over the range of 15 to 150 ng/L (ppt). The recoveries were above 70% except when the silica gel/charcoal cleanup procedure was used (Table 6). The reagent water and wastewater recovery data were nearly identical. The contractor's final report is available through NTIS. [11] The detection limit of 3 ng/L is based on a signal at 5x the noise level assuming a 5 µL injection of a 1 mL extract from a 1 L sample that exhibits no recovery losses.

TABLE 6

INITIAL METHOD VALIDATION: Reagent Water and Wastewater

Matrix[a]	Spike Level (ng/L)	n	Percent Recovery: No Cleanup Column[b]	After 1 Cleanup Column[c]	After 2 Cleanup Columns[d]	After 3 Cleanup Columns[e]
Reagent Water	100	5	93 ± 2.6	85 ± 2.3	77 ± 12	38 ± 8.2
Industrial Wastewater	20	1	--	63	--	--
	20	1	--	71	--	--
	40	1	--	70	--	--
	80	1	--	75	--	--
	100	3	--	--	--	43 ± 6.8[e]
Municipal Wastewater	15	7	--	84 ± 5.1[f]	--	--
	150	7	--	82 ± 4.2[f]	--	--

a) No 2378-TCDD found in any control samples at a detection limit of 3 ng/L
b) Silica gel column
c) Silica gel then alumina column
d) Silica gel then alumina then silica gel/charcoal column
e) HRGC/ECD results
f) alumina column only
n) number of replicates

PUBLIC COMMENT

The method was published as a proposed test procedure with Methods 601-612, 624 and 625 in the December 3, 1979 issue of the Federal Register and as such was subject to a period of public comment to proposed rule-making. Only single lab data was available at that time. All the proposed methods were to undergo an interlaboratory validation study involving 15 to 20 labs before the methods were published in the final rulemaking which is expected in the Fall of 1982. The interlaboratory results for Method 613 are the topic of the next paper. [12]

The initial public comment period to the proposed rulemaking was extended to April 28, 1980. On January 13, 1981, at the request of the Chemical Manufacturing Association, the public comment period was reopened until February 2, 1981. The first round of public comment questioned the need for screening by GC/ECD. The scientific community stated that the procedure should use only GC/MS during any assay step and that the internal standard should be added to the original sample. This would make all samples potentially hazardous, because each sample would contain the labeled 2,3,7,8-TCDD.

The second round of public comment reflected the advances in the state of the art for 2,3,7,8-TCDD analyses that occurred in the summer and fall of 1981. The use of reverse phase and normal phase HPLC was shown to give isomer specific separation of 2,3,7,8-TCDD.[13] This high resolution cleanup procedure was reported to be effective in determining ng/L amounts of 2,3,7,8-TCDD in human milk [14] and particulate samples.[15] Achieving isomer specific separation prior to the assay step obviates the need for capillary column gas chromatography which still had not demonstrated an isomer specific separation of 2,3,7,8-TCDD. This high resolution HPLC cleanup technique appears too labor intensive for use in a routine commercial laboratory.

During this time a comprehensive method for determining 2,3,7,8-TCDD at the parts per trillion level in human milk, fish, water, soil, and sediment was reported.[16] The original sample was always fortified with labeled 2,3,7,8-TCDD with this method. Basic and acidic coextractants were removed using 1N KOH and concentrated sulfuric acid. Woelm alumina (neutral grade, activity I) and Florisil were used for cleanup. Extracts were taken just

to dryness and dissolved in 60 µL of benzene for HRGC/HRMS analysis. A 30 m x 0.25 mm glass capillary column coated with SE-30 was used for chromatographic separation and was interfaced to a high resolution mass spectrometer (HRMS) that differentiated any other ions that were 30 millimass units larger or smaller than molecular ions of 2,3,7,8-TCDD. The selectivity of the SE-30 capillary column was judged equivalent to OV-101 capillary columns which had previously been shown to coelute at least two other TCDD isomers with 2,3,7,8-TCDD.[17] The requirements for achieving confirmation of the presence of 2,3,7,8-TCDD included those listed above for Method 613, plus two additional criteria:

1) the signals must be greater than 2.5x the noise level, and

2) correct response must be obtained after all extracts are fortified with authentic 2,3,7,8-TCDD and internal standard.

The latter criterion demands two HRGC/HRMS runs per extract, the raw extract and the extract after fortification. The extremely small volume of the final extract and the requirement for HRMS removes the final steps of this method from the repertoire of the routine commercial analytical laboratory.

The most serious fault in the early version of Method 613 was a lack of isomer specificity. A Silar 10C capillary column (55 m x 0.25 mm ID) was reported to give the unique separation of 2,3,7,8-TCDD from the other 21 TCDD isomers.[18] Some of the public comment deprecated the utility of this column. Because of temperature restrictions, it was apparent that no penta-, hexa-, hepta-, or octachlorodibenzo-p-dioxins would elute from this highly polar column. This constraint is irrelevant for a method that contains a single specific isomer as the only target compound.

FINAL METHODS DEVELOPMENT

The interlaboratory methods validation studies mentioned above are conducted by the Quality Assurance Branch of EMSL-CI. Monsanto Research Corporation was awarded the contract for the Method 613 validation study in the Spring of 1980. The Method Detection Limit (MDL) and Analytical Curve for Method 613 were determined by Monsanto.[19] Below 100 ng/L the initial version of Method 613 performed very well and an MDL of 6 ng/L was established (Table 7). The higher dosing levels used to determine the analytical curve (working range) demonstrated a trend toward 50% recovery as the concentration approached 1000 ng/L. At high concentrations, there may not be a sufficient volume of extracting solvent to remove all the 2,3,7,8-TCDD from the aqueous sample. A brief study indicated that at a high dosing level there was a significant amount of 2,3,7,8-TCDD left in the aqueous phase after extracting with three 60 mL aliquots of methylene chloride. This problem still needs more exploration; however, this conclusion is not consistent with the results in Table 4.

TABLE 7

INITIAL METHOD: Reagent Water MDL and Analytical Curve

Spike Level (ng/L)	Number of Replicates	Mean Result (ng/L)	MDL[a] (ng/L)
15	7	14.3 ± 1.89	5.9
3	2	Not detected	--
60	2	60.5 ± 3.5	--
600	2	310 ± 71	--
1000	2	495 ± 7.1	--

a) Requires at least 7 replicates MDL = std. dev. * Students' t. (= 0.001)

REVISION OF THE METHOD -- The sample processing steps of the initial method were cumbersome. After the preliminary extraction, the analyst had to manipulate 200 mL of the organic phase. During the acidic and basic extractions, the methylene chloride phase, which was to be retained, was at the bottom of the separatory funnel. In view of the public comment and at the advice of Monsanto Research Corporation, the sample processing steps were revised. The GC/ECD screening step and the charcoal-silica gel cleanup column were deleted. The charcoal/silica gel cleanup is appropriate for sediment and soil samples, but not for water samples. The sample processing was revised to include:

1) Addition of the internal standard to every sample prior to extraction,
2) a solvent exchange to hexane immediately after the initial extraction;
3) washing with 10 N NaOH and concentrated H_2SO_4; and
4) using a final extract volume of 0.2 to 1.0 mL.

The criteria for qualitative identification were expanded to include using a 60-m Silar 10C (or equivalent) capillary column to obtain isomer specificity and requiring that all peaks be greater than 2.5x the noise level. The Quality Assurance requirements were expanded to include calculating the recovery of the internal standard and flagging the results as suspect if the internal standard exhibited less than 50% recovery at the assay step. This is a check on extreme matrix effects and errors during sample processing. The final version of the method is presented as a flow chart in Figure 4 and is the method used during the interlaboratory validation study. The data summarized in Tables 1 through 5 are still appropriate to the revised method

because the data describe performance at individual steps in the method.[a] The steps are the same, only the order in which they are performed was revised.

The entire revised method was validated in reagent water, an industrial wastewater, and a municipal wastewater (Table 8). The dosed reagent water samples exhibited an MDL of 2 ng/L based on the standard deviation of seven replicates dosed at 5 ng/L and processed through the method. This is reasonable compared with the not detected results for the 3 ng/L spike in Table 7 because the final volume is now 0.2 mL, not 1.0 mL. The industrial wastewater gave results equivalent to reagent water. The municipal wastewater results showed acceptable accuracy (-8% bias) and precision (20% RSD) even though a matrix effect was evident. The small amount of bias would have been much larger had the internal standard been added after the extraction step as was specified in the earlier version of Method 613. The 50% criterion for the percent recovery of the internal standard may be too rigorous a cutoff for a QA validation check. In this case, the recovery of internal standard averaged 14%, which requires that the results be flagged as suspect, even though the accuracy showed only a -8% bias.

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[a]During the interlaboratory study, 2 of the 11 labs experienced a total loss of 2,3,7,8-TCDD at the alumina column cleanup step.[10] This indicates that the activity of the specified alumina may not be uniform between batches and lots and emphasizes the importance of using Laboratory Control Samples (LCS). The method specifies that any lab using the method must analyze 4 LCS samples and demonstrate acceptable proficiency prior to analyzing any environmental samples.

TABLE 8

REVISED METHOD VALIDATION: Reagent Water and Wastewater

Matrix	Spike Level (ng/L)	Number of Replicates	Mean Result (ng/L)	MDL[a] (ng/L)	% Recovery of Internal Std.
Reagent Water	5	7	4.77 ± 0.51	1.6	87 ± 23
Industrial Wastewater	5	3	4.29 ± 0.03	-	104 ± 25
Municipal Wastewater	25	3	23.1 ± 4.7[b]	-	14 ± 6

a) Requires at least seven replicates: MDL = std. dev. *Students' t (=0.001)
b) Recovery of labeled internal standard less than 50%

SUMMARY

The final version of Method 613 is somewhat typical of the 600 series of Methods (604-611) for solvent extractables except that the labeled internal standard is added to every sample. The aqueous sample is extracted with methylene chloride, which is then exchanged to hexane which is then washed with 10N NaOH and concentrated to H_2SO_4 to remove acidic and basic coextractants and any neutral compounds which easily react with concentrated sulfuric acid. The assay step is performed using a capillary column that gives a unique isomer specific separation for 2,3,7,8-TCDD [12] and is coupled to a MS detector operating in the SIM mode. The sensitivity requirement demands that the exit end of the capillary column be positioned as close to the ion source as practical. Two cleanup procedures are provided to help separate 2,3,7,8-TCDD from interferences. Criteria for the qualitative confirmation of the presence of 2,3,7,8-TCDD are:

1) The GC column is isomer specific.

2) The native 2,3,7,8-TCDD and labeled 2,3,7,8-TCDD internal standard coelute.

3) The chlorine isotope ratio (m/z 320/322) is within ± 10% of the calibration standard.

4) All peaks are greater than 2.5x the noise level.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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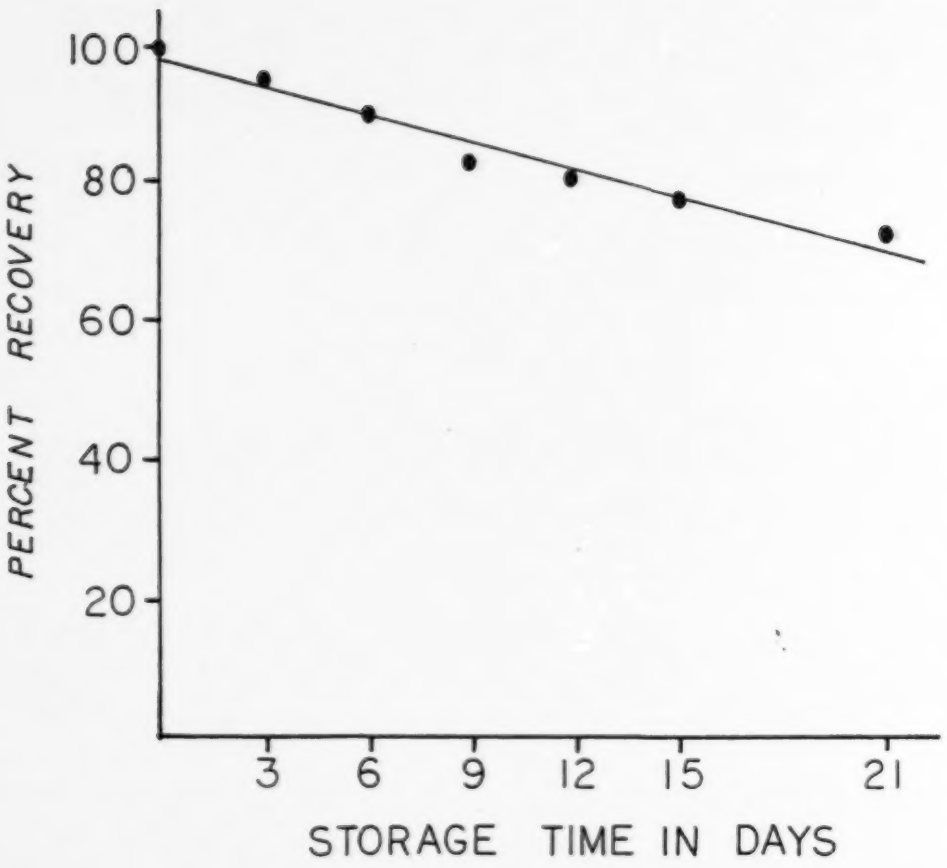

Figure 1. Recovery of 2,3,7,8-TCDD from Chlorinated Water.

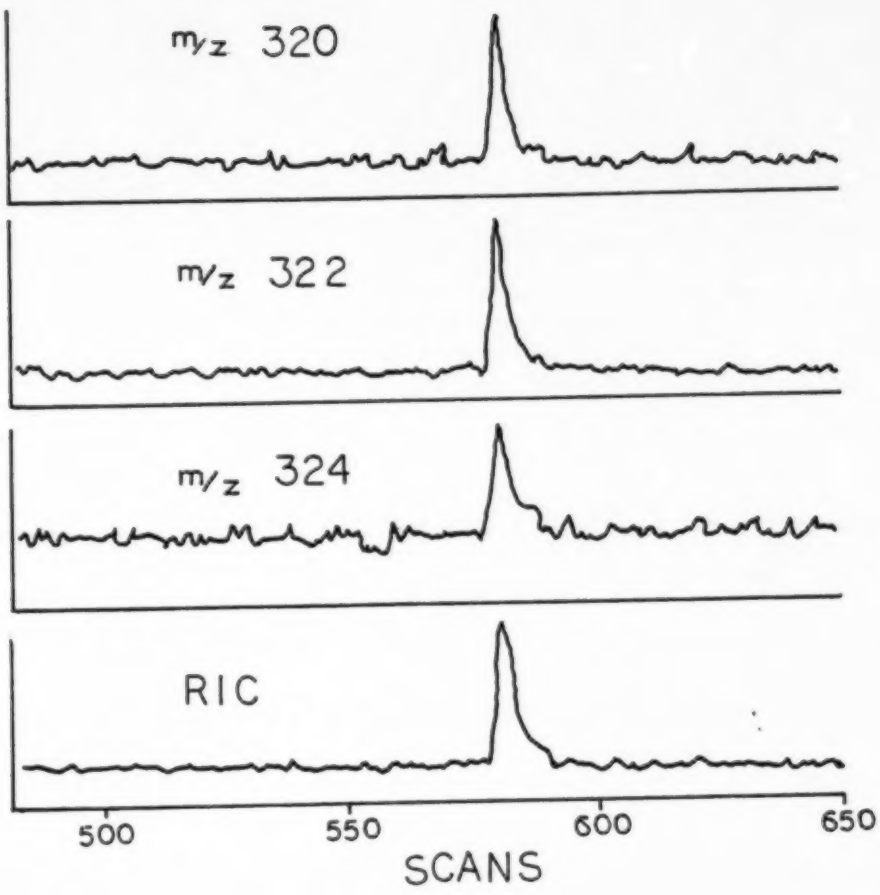

Figure 2. SP-2250 Capillary Column Performance for 2,3,7,8-TCDD.

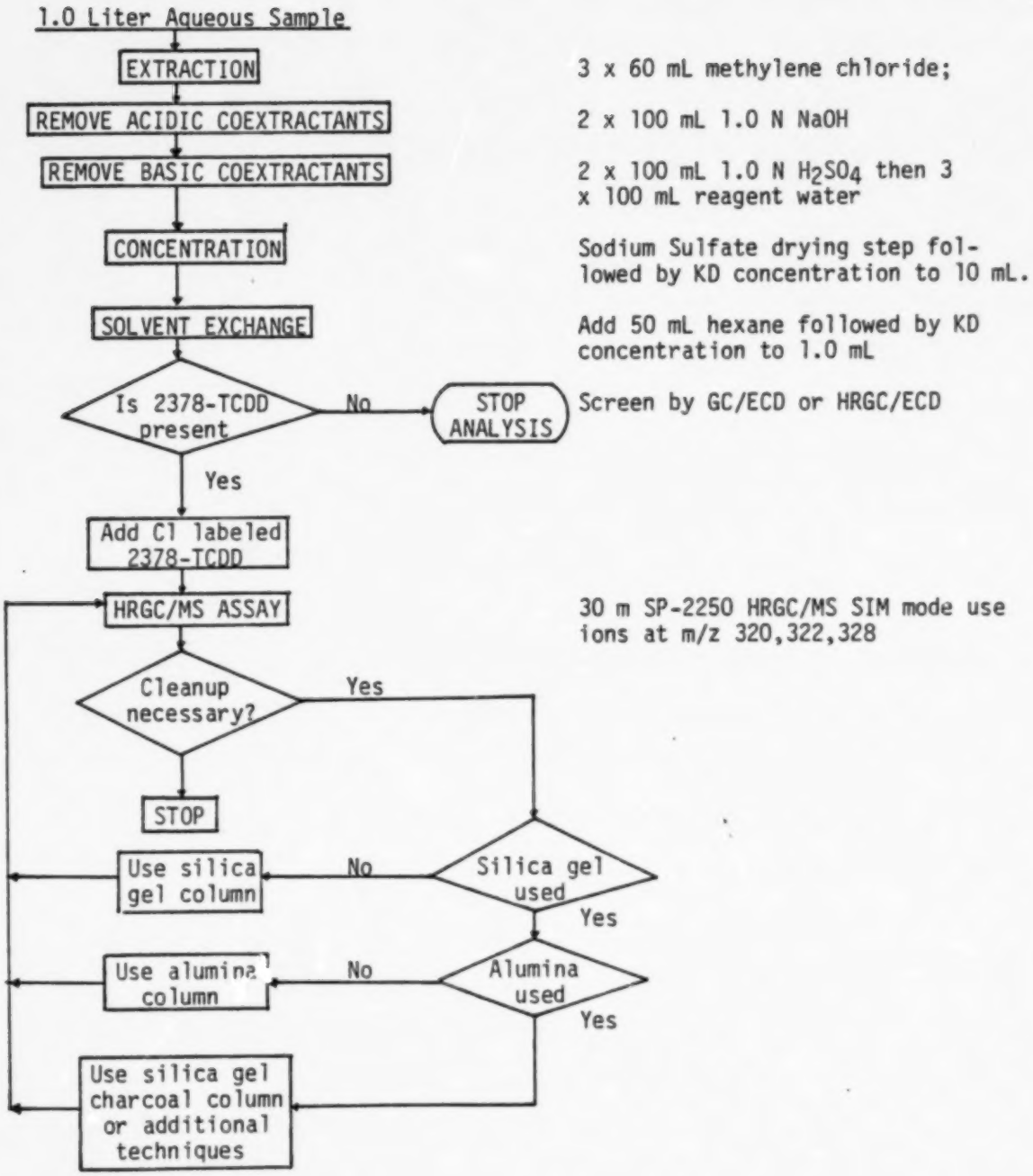

Figure 3. Flow Chart of Initial Method.

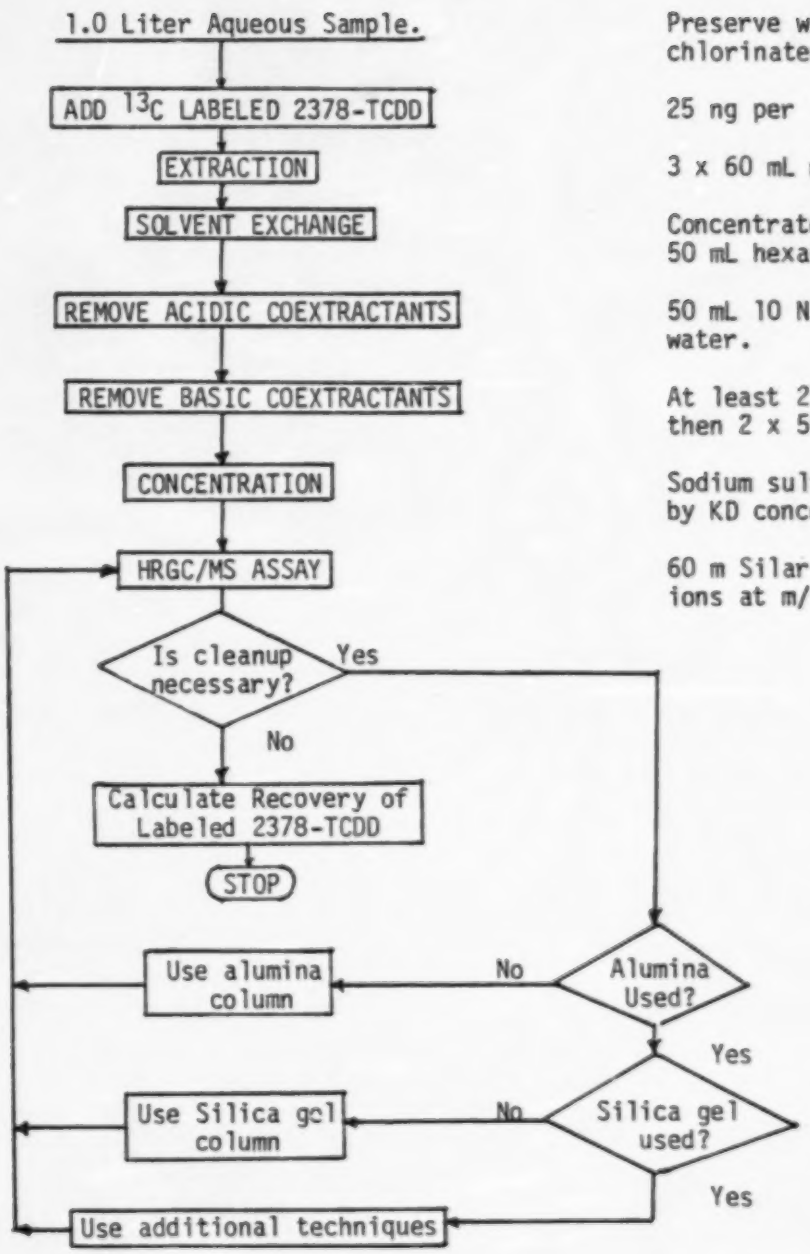
1.0 Liter Aqueous Sample.
Preserve with sodium thiosulfate if chlorinated.
ADD ^{13}C LABELED 2378-TCDD
25 ng per liter.
EXTRACTION
3 x 60 mL methylene chloride.
SOLVENT EXCHANGE
Concentrate to 1.0 mL by KD then add 50 mL hexane, concentrate to 1.0 mL.
REMOVE ACIDIC COEXTRACTANTS
50 mL 10 N NaOH then 50 mL reagent water.
REMOVE BASIC COEXTRACTANTS
At least 2 x 50 mL conc H_2SO_4 then 2 x 50 mL reagent water.
CONCENTRATION
Sodium sulfate drying step followed by KD concentration to 0.2 to 1.0 mL.
HRGC/MS ASSAY
60 m Silar 10 C HRGC/MS SIM mode use ions at m/z 320,322,332
Is cleanup necessary?
Yes
No
Calculate Recovery of Labeled 2378-TCDD
STOP
Use alumina column
No
Alumina Used?
Yes
Use Silica gel column
No
Silica gel used?
Yes
Use additional techniques

Figure 4. Flow Chart of Final Method.